Biofouling attractants from a brown marine alga *Ecklonia cava*

M. Sidharthan, G.S. Viswanadh, Kyoung Ho Kim, Hyuk Jun Kim and H.W. Shin*

Department of Marine Biotechnology, Soonchunhyang University, Asan City-336 745, South Korea

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**Abstract:** In recent years, industrial pollutants and the mountain forest fire ashes released into seawater cause damage to the marine environment, mainly it reduces the algal productivity in the inter tidal region. To get recover from the stress due to pollutants and to increase the growth and development of biofouling algae (benthic organisms), *Ecklonia cava* extract was investigated for its biofouling attracting efficiency. Bioactive guided fractions of *E. cava* extract derived from column chromatography were tested against spore attachment of a fouling alga, *Ulva pertusa*. Fraction B showed increased spore attachment rate with a maximum of 92±5%. This fraction was further analysed on HPLC, GC-Mass and NMR, deduced as pentadecanoic acid.

**Key words:** Biofouling attractant, *Ecklonia cava*, Spore attachment, *Ulva pertusa*

**Introduction**

The biofouling phenomenon is well known in ecological system of marine environment (Evans, 1981). Rapid growth in industrial activities along the Korean coast increased the pollutants load into coastal waters and it considerably changed the macrobenthic (biofouling) animals and algal communities, which are harbouring rich population of free living marine forms. Inorganic pollutants present in industrial wastes and forest fire ashes tend to bioconcentrate in marine organisms (Shin et al., 2002). Excessive inorganic pollutants bioconcentrated in algae affect the growth and spore generation. In recent years, artificial reefs have been employed to increase the productivity of marine forms. These artificial reefs are coated with fouling attractants to increase the settlement of reproductive materials (larvae, spores etc.) and successful metamorphosis. As spore attachment is a critical stage in algal growth and development it was employed in biofouling investigations (Fletcher and Callow, 1992; Hellio et al., 2001).

Several bioactive compounds like, diterpene lactone like ecklonialactone A and B, eckol, dieckol and arseno betaines and their metabolites are reported from *Ecklonia* sp. (Fukuyama et al., 1985; Blunden and Gordon, 1986; Edmonds et al., 1987; Shibata et al., 1987; Kurata et al., 1989). Recently, methanol extract of *Ecklonia stolonifera* has shown to possess potent antioxidant activity (Kang et al., 2004). Although many secondary metabolites and their specific activities reported from *Ecklonia* sp., their biofouling attracting efficiency remains unexplored. During our search on biofouling attractants from marine natural products, we investigated *E. cava* for its spore attracting efficiency and the responsible chemical constituent was characterised.

**Materials and Methods**

A brown alga *Ecklonia cava* was collected from Busan, south coast of South Korea and transported to laboratory. The shade dried algal material (500 g) was exhaustively extracted with methanol (2 liter) in a soxhlet extraction apparatus for 24 hr. The solvent was removed under reduced pressure, the brown colored concentrate (2.1 g) was adsorbed on silica gel and column chromatographed over silica gel. The column was successively eluted with n-hexane : ethyl acetate mixtures by increasing the polarity. Elution was monitored by TLC, while collecting 250 ml of fractions. The combined major fractions (A-E) were subjected to bioassay against the spore attachment of a fouling alga *Ulva pertusa*. Bioassay guided fraction [B, eluent n-hexane: ethyl acetate (3:2)] was subjected to purification over silica gel chromatography, HPLC (Shimadzu LC-6A: using a C18 MS column) and the isolated compound was identified through its GC-MS (Shimadzu GC-10-AT QP5050 A; FID; HP-5 column - 30m) and 13C NMR (200MHz) (Bruker) and 1H NMR (50 MHz) with spectral analysis.

Thalli of the green fouling alga *U. pertusa* was thoroughly washed with 0.2 µM filtered seawater and placed in dry petridishes under fluorescent light at room temperature for overnight. After drying, the algal material was covered with filtered seawater to facilitate the release of motile cells. In the resulting spore suspension, spore density was adjusted to 30,000 spores ml-1. From the column fractions (A-E), about 5 mg was dissolved in 0.5 ml DMSO and 0.5 ml of distilled water to prepare a desired concentration (100 mg l-1) of solution. Into 2% agar gel, test extracts (50-75 µl) were loaded and it was evenly spread about 2x2 cm area on the acid clean glass slides. Agar coated film without the test compounds served as control. The agar coated slides were exposed to *U. pertusa* spore suspension for 10 hr under dark condition. Later, the slides were gently washed with distilled water and number of spores attached per mm2 was counted under microscope (Olympus BH2, 400 x magnifications).
Table 1: Column chromatography fractions of Ecklonia cava

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluent</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>H</td>
<td>Yellow oil, Fr.A</td>
</tr>
<tr>
<td>9-22</td>
<td>H+ 5%EA</td>
<td>Intractable gum</td>
</tr>
<tr>
<td>23-36</td>
<td>H+10%EA</td>
<td>Yellowish gum, Fr.B</td>
</tr>
<tr>
<td>37-44</td>
<td>H+20%EA</td>
<td>Yellowish gum, Fr.C</td>
</tr>
<tr>
<td>45-59</td>
<td>H+30%EA</td>
<td>Intractable gum</td>
</tr>
<tr>
<td>60-68</td>
<td>H+50%EA</td>
<td>Green gummy solid, Fr.D</td>
</tr>
<tr>
<td>69-74</td>
<td>H+75%EA</td>
<td>Green gummy solid, Fr.E</td>
</tr>
<tr>
<td>75-81</td>
<td>100%EA</td>
<td>Intractable gum</td>
</tr>
</tbody>
</table>

(H: n-hexane; EA: Ethyl acetate)

Results and Discussion

Methanol extract of *E. cava* showed five major spots with long streaks on TLC [n-hexane : Ethyl acetate(4:1)], were subjected to column chromatography. The eluted fraction details are given in Table 1. The major fractions (A-E) were subjected to spore attachment bioassay using a fouling alga *U. pertusa* (Fletcher and Callow, 1992; Shin and Smith, 2001). The spore attachment rates estimated for different fractions are given in Fig. 1. The data mentioned is mean (%) ±S.D of the three independent assays at 100 mg l⁻¹ concentration.

It is evident that all the fractions showed efficient fouling attraction character towards *U. pertusa* spores (Fig. 1). But the fraction B considered being more effective in attracting spore attachment (92±5%) and fraction E was comparatively less. Thus the effective fraction B was further analysed for responsible constituents.

![Fig. 1: Effects of different fractions of *Ecklonia cava* on attachment of *Ulva* spores (Mean % ± SD)](image)

The fraction B was repeatedly chromatographed over silica gel and separated on HPLC as low melting solid. This compound showed a molecular ion m/z at 242 in GC-MS spectrum and other fragment ions at m/z 199, 185, 157, 143 etc., reveals that long chain carboxylic moiety (Fig. 2). The signals appeared in ¹H NMR spectrum at δ 0.98 as triplet, δ 1.21-1.28 as broad singlet and at 2.41 as triplet correspond to the methyl and methylene signals of long chain moiety (Fig. 3a). Further, the signals appeared in ¹³C NMR spectrum at δ 181 indicating the presence of acid function as well as methylene and methyl signals resonated are well in agreement with the expected values (Fig. 3b). The above spectral data confirmed the isolate as pentadecanoic acid. The present data is also well in agreement with the earlier reports from marine algae (Kamenarska *et al.*, 2002; Carballera and Miranda, 2003) and literature (Pouchert and Behnke, 1992). Thus, we conclude that the constituent from *E. cava* responsible for biofouling attraction was pentadecanoic acid.

![Fig. 2: GC-MS spectrum of purified fraction B collected from silica gel column chromatography (eluent: n-hexane : ethyl acetate) of *Ecklonia cava* extract](image)
Fig. 3: NMR spectra of purified B (eluent: CDCl₃) of Ecklonia cava extract (a: ¹H NMR, b: ¹³C NMR)
acid. In an experimental study employing test coatings (1 mM) with a range of saturated fatty acids (C_12-20) showed that the spore attachment increased with the presence of number of carbon atoms (Callow and Callow, 2000). In which pentadecanoic acid (C_15) exhibited about threefold increase in Enteromorpha spore attachment. This is in agreement with the present study. Similarly fatty acids have also been implicated as inducers of larval settlement and metamorphosis (Jensen, 1990).

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References


